

A REVIEW AND STUDY OF BALLAST WATER CONTAMINANTS

By

LINDA ASBURY LORE

A THESIS PRESENTED TO THE GRADUATE SCHOOL  
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT  
OF THE REQUIREMENTS FOR THE DEGREE OF  
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

1998

## ACKNOWLEDGMENTS

No good and successful project can be conceived and carried out without help and support. From inception to implementation, Dr. Rodrick provided the guidance necessary for success. Dr. Tiffany's on site assistance, both with specimens and advice, was invaluable. Pertinent suggestions and encouragement were provided by Dr. Balaban.

Dr. Rodrick's laboratory and assistance were helpful and supportive with this project. Technical assistance from the University of Florida Soils Laboratory was instrumental in providing necessary data.

Acquiring the necessary specimens required not only scientific expertise, but a lot of travel by my friends and family. I would like to thank my sister Beth for her patience and assistance in the preparation of this project. Without the support of my mother and father, the project would have never gone as smoothly.

## TABLE OF CONTENTS

ACKNOWLEDGMENTS .....	ii
ABSTRACT .....	vi
INTRODUCTION .....	1
REVIEW OF LITERATURE .....	3
Movement of Exotic Species .....	3
Effect of Ballast Water on Movement of Exotic Species .....	17
Methods and Regulation for Ballast Water .....	34
Review of Work From Other Institutions .....	39
Ireland .....	39
United States .....	42
Objectives .....	46
MATERIALS AND METHODS .....	48
Source of Ballast Water .....	48
Collection of Ballast Water .....	48
Microbiological Analysis .....	48
Invertebrate Analysis .....	50
Turbidity Analysis .....	50
Other Analyses .....	50
RESULTS AND DISCUSSION .....	51
Microbiology Analysis Results .....	51
Mayport, Florida .....	51
St. Petersburg Harbor, Florida (Puerto Rican Samples) .....	51
St. Petersburg Harbor, Florida (Caribbean Samples) .....	52
Tampa Bay, Florida .....	52
Port Manatee, Florida .....	53
CONCLUSION AND RECOMMENDATIONS .....	55
LITERATURE CITED .....	57
BIOGRAPHICAL SKETCH .....	61

## LIST OF TABLES

Table	Page
1. Specification of ballast water capacities and distributions in six vessel types...	11
2. Probability of aquatic organisms and pathogens surviving from transfer to different water salinities.....	23
3. Imports, exports, and number of ships passing through twelve ports in Ireland.	40
4. Number of vessels visiting Dublin Port, Ireland in ballast.....	41
5. Number of vessels visiting Cork Harbor, Ireland in ballast.....	41
6. Number of vessels visiting Limerick Port, Ireland in ballast.....	42
7. Number of vessels visiting Waterford Port, Ireland in ballast.....	42
8. Monthly ship arrivals in ballast in five southern ports of the United States.....	44
9. Last port of call by region for ships in ballast from foreign ports in Charleston, S. C.....	44
10. Last port of call by region for ships in ballast from foreign ports in Savannah, GA.....	45
11. Last port of call by region for ships in ballast from foreign ports in Miami, FL.....	45
12. Last port of call by region for ships in ballast from foreign ports in Tampa Bay, FL.....	45
13. Last port of call by region for ships in ballast from foreign ports in New Orleans, LA.....	46
14. Microbiological results of ballast water from Mayport, FL.....	51
15. Microbiological results of ballast water from St. Petersburg Harbor, FL., (Puerto Rican samples).....	51

16. Microbiological results of ballast water from St Petersburg Harbor, FL., (Caribbean samples).....	52
17. Microbiological results of ballast water from Tampa Bay, FL.....	52
18. Microbiological and metal analysis results of ballast water from Port Manatee, FL.....	53

Abstract of Thesis Presented to the Graduate School of the University of Florida  
in Partial Fulfillment of the Requirements for the Degree of Master of Science

## A REVIEW AND STUDY OF BALLAST WATER CONTAMINANTS

By

Linda A. Lore

December, 1998

Chairman: Gary E. Rodrick

Major Department: Food Science and Human Nutrition

Ship ballast water has been suggested as a vector in the dispersal of non-indigenous marine species all over the world for the last 90 years. In June of 1988, a heightened awareness of the potential danger of introducing non-indigenous species by ship ballast water was brought about by the discovery of the Eurasian zebra mussel (*Dreissena polymorpha*) in Lake St. Clair and Lake Erie. In addition, during the summer of 1991, toxigenic *Vibrio cholerae* 01 resembling the Latin American strain was discovered in oysters and in cargo ship ballast water in Mobile Bay, Alabama, and was a direct threat to the shellfish harvesting waters along the Florida coast. Along with an extensive literature review, this study examined ship ballast water data collected from ships entering four Florida ports. Ballast water was examined for the presence of bacteria, invertebrates, and metals. Harmful microorganisms, invertebrates, and metals were found in samples taken for this study.

Due to the discovery of harmful microorganisms, invertebrates, and metals in the samples taken from Florida ports, research and risk management analyses should be

performed in the future. Funding for research of ship ballast water should be provided, as well as legislation for easy access of samples for further research.

## INTRODUCTION

Non-indigenous species have been introduced to new locations worldwide for many years and recently problems have occurred from these introductions. Most of these introductions have been accidental, while others have been deliberate (Sheehan, 1995). An introduced or non-indigenous species can be defined as any species intentionally or accidentally transported and released by humans into an environment outside its present range (Sheehan, 1995). Significant invasions have occurred in Australia including several toxic dinoflagellates and other potentially harmful exotic species. These introductions have resulted in major ecological and economic consequences. There have also been serious concerns over effects on human health, fishing, and aquaculture industries (Rigby et al., 1995). There have also been concerns of non-indigenous organism introduction in Europe. No monitoring of ballast water exchange is practiced in Europe as well as throughout much of the maritime industry (Sheehan, 1995). The discovery in June and July of 1988 of the Eurasian zebra mussel Dreissena polymorpha in Lake St. Clair and Lake Erie of the North American Great Lakes became a focal point of interest in aquatic biological invasions in the United States (Carlton et al., 1994). Thirty months after the zebra mussels were found, the U.S. Congress passed Public Law 101-646 (November 29, 1990), the "Non-indigenous Aquatic Nuisance Prevention and Control Act of 1990" (Carlton et al., 1994). Unfortunately, invasions may still be occurring.

In January 1991, epidemic cholera was reported in Peru and rapidly spread through Latin America and into Mexico (McCarthy and Khambaty, 1994). In July and



September, 1991 and June, 1992, toxigenic Vibrio cholerae 01 was isolated from five oysters and a mackeral fish off the coast of Mobile Bay, AL. The organism was also found in the ballast water of five cargo ships docked in ports along the U.S. Gulf of Mexico (McCarthy and Khambaty, 1994). This is a major threat to the Florida coastline. For this research project, samples have been taken from four area ports and analyzed for microorganisms, invertebrates, and metals. This was done in order to determine if the state of Florida is at risk of invasions by non-indigenous species. An extensive literature review was also performed to address the issue on a much broader base.

## LITERATURE REVIEW

### Movement of Exotic Species

Ocean going vessels have always been recognized as dispersal agents for living organisms. Today, distributions of thousands of species of plants, fungi, molds, bacteria and viruses, nematodes, earthworms, insects, spiders, millipedes, mites, ticks, snails, slugs, mammals, along with many others, can be explained in terms of human colonization (Carlton et al., 1994, Carlton, 1985, 1987, 1989). According to Carlton, there are three major types of watercraft that are now used on the world's oceans. These include the following:

1. Passenger vessels (passenger liners, ferries, and excursion boats)
2. Cargo vessels (bulk carriers, container ships, and tankers)
3. Specialized vessels (barges, fishing vessels, semi-submersible exploratory drilling platforms, and warships).

A ship can be considered as a "biological island." Living organisms can occur both on the outside and on the inside of a vessel (Carlton et al., 1994).

Organisms on the outside of the vessel are considered fouling organisms. "Bio-fouling" occurs on the hull, rudder, propellers, anchor, and any underwater structure. The length of time it takes for fouling depends on the resistance or susceptibility of the exposed area to larval settlement and recruitment and the length of time the surface area has been exposed, among other factors. Bacteria are usually the first to colonize followed

by diatoms, fungi, and blue-green algae (cyanophyte bacteria). Together, these colonizers make up the “primary film” which is necessary for larger fouling organisms to settle. Barnacles and algae can settle on submerged surfaces before the primary film. Hull surfaces can develop large fouling communities of seasquirts, hydroids, and seaweeds, which at times may be over a foot thick. Anchors and chains left in the water too long can become fouled. When exposed to the air most of the fouling organisms die, however, waves splashing on the anchors and chains can prolong life even long enough to be dropped in again at a different location. There are three types of fouling organisms: those that are attached, those that are associated with the attached organisms, and those that are passively entrained by the vessel. Entrained organisms are those that have been entangled or caught up on structures external to the vessel (Carlton et al., 1994; Carlton, 1985; 1987; 1989; Young, 1994).

Another type of organism that occurs on the outside of a vessel is the boring organism. This organism attacks wooden structures below the waterline. Wood borers include shipworms, boring clams (pholads), and amphipods (Chelura). The outside of the vessel has provided the main transport for the dispersal of marine organisms. However, there have been changes in shipping that suggest that dispersal of fouling organisms through vessel transport has declined. These include:

1. Increased vessel speeds leading to organisms being washed off the vessel.
2. Increased use of toxic anti-fouling paints.
3. Decreased port residency leading to decreased colonization.
4. Increased frequency in cleaning the hull.

Other phenomena suggest that dispersal of fouling organisms through shipping still occur quite commonly. Fouled vessels still travel the ocean, therefore, continuing to spread the organisms. Further, slow moving ships still travel the ocean spreading organisms. Investment in ship maintenance has decreased. Some of these fouling organisms have grown tolerant to the copper-based anti-fouling paints and can lead to more invasions in the future. Lastly, ballast water can transport larval, juvenile, and adult stages of fouling organisms (Carlton et al., 1994; Young, 1994).

Transportation of organisms inside the vessel happens quite frequently and the range of the types of organisms is wide. Internal sites for fouling organisms include the sea chest, seawater pipe systems, and intakes. According to Carlton et al., (1994), there are ten types of water that can be brought onto the ship with the potential for transporting organisms. These include:

1. Chain locker water (water brought on board with anchor chains)
2. Bilge water (water collected in the bilge)
3. Potable water (drinking, bathing, and galley water)
4. Engine cooling water (water used to cool the main power plant of the ship)
5. Sanitary system water (sewage water that can carry bacteria, protozoans, nematodes, human viruses, and helminths)
6. Live well water (used in holds dedicated to keep fish, shellfish, or bait alive)
7. Ballast water (discussed later)
8. Fire control water (held in fire control lines)
9. Propeller shaft cooling water (in some ships, water is taken on to cool the propeller shaft)

10. Incidental water (waves and sea-spray breaking over and on the ship).

Planktonic organisms can get into these water systems and be transported to any ships' destination (Carlton et al., 1994; Carlton, 1985).

Carlton et al., (1994) listed four categories of organisms that can be found on board vessels. Planktonic organisms found in incidental water can be washed on board the ship through waves and ocean sprays. Benthic organisms can be captured by fishing vessels within nets, trawls, and grabs and remain on deck for a long period of time until transported many miles away from where they were caught. Living fish and shellfish are brought on board for human consumption and can be discarded while still living in foreign waters. Some organisms are carried on board as pets by crew. These can also be released into environments where they did not originate. A prime example of this is the release of the horseshoe crab in the North Sea (Carlton et al., 1994; Carlton, 1985).

Sediments, which include mud, sand and fractions of rock, can accumulate inside a ship in many places such as holds, wells, tanks, and lockers. Chain lockers may contain sediments because of neglect in washing, and therefore remain damp within the locker. It is possible that if there is sufficient mud and water along with humid conditions, some invertebrates can survive. These invertebrates include dinoflagellates, nematodes, ostracods, and many other species in their resting stages (Carlton et al., 1994; Hallegraeff and Bolch, 1991; 1992).

"Dry ballast" containing rocks, sand, trash, detritus, soil, and other solid material are known to be responsible for transporting living organisms from one place to another. Today, dry ballast is used very little if at all. Packing materials or "dunnage" was also used in the past to protect cargo. Dunnage consisted of terrestrial grasses, marsh grasses,

sea-grasses, dried seaweed, mats, boughs, rattans, and woods. These materials contained many live organisms including plants, plant seeds, insects, spiders, other arthropods, earthworms, and snails. Naturally occurring dunnage is not used anymore in shipping, but native peoples from coastal countries might still use this method of ballast (Carlton et al. 1994; Carlton, 1985; 1987; 1989).

The use of water to ballast a ship is the main method for keeping the ship stable in the ocean (Carlton, 1985). A modern oceangoing ship is basically a floating steel box that is shaped and equipped by man to carry thousands of human lives or thousands of tons of cargo. It must combine strength and flexibility, great power and the precise balance to roll with the punches of the sea (Lewis and O'Brien, 1965). Therefore, there are several reasons why ballast water is taken on board the ship.

1. To diminish stress: Ballast water when properly distributed helps counteract forces on the hull.
2. To provide proper stability and trim: Ballast water is used for trimming (to control front and back angle), for stabilizing (to control side to side angle) using stability tanks to control roll, to reduce free surface area in the tank in order to reduce the rocking back and forth of the water in the tank, to minimize the beating of a vessel while at sea.
3. To aid in propulsive efficiency: Ballast water controls the level of the propeller and the bow thruster in the water, which helps in controlling propulsion.

4. To aid in maneuverability: Ballasting brings the ship lower in the water which reduces freeboard exposure to the wind; adjustment of trim and list helps in maneuvering.
5. To compensate for the consumption of fuel and potable water: Ballast water provides added weight when fuel and water are consumed.
6. To provide for operational needs (proper draft): In many ports and coastal industries there are requirements that ships have to follow. Requirements to have more ballast on board in order to get under loading cranes or chutes, or bridges might be necessary.
7. To provide for increased comfort at sea under weather conditions: Taking on ballast water helps reduce the roll of the ship in bad weather conditions.
8. To clean decks and holds: ballast water, especially freshwater ballast, can be used to wash down decks and holds.

“Ballast condition” directly affects the performance of the vessel. When the ship takes on too much ballast water it is considered to be in “stiff” condition. This makes the ship heavy allowing for the potential of speed loss. When the ship does not bring enough ballast water on board it becomes “cranky” or “tender” and the potential for capsizing is great (Carlton et al., 1994; Carlton, 1985).

Ballast water is brought on board a vessel from several feet below the water surface using dedicated pumps. These same pumps and external hull openings are used to bring water in and remove water from the vessel. Water is gravitated or pumped in or out of a particular tank or hold but not necessarily the same hold. Tanks located above the waterline require the water to be pumped in but emptied by gravitation. Tanks

located below the waterline are filled by gravitation but have to be emptied by pumping. There are some ships that have automatic ballasting systems. Container ships have probably the most advanced ballasting operations using computers. The pump rates vary among ships but average varies from  $75\text{m}^3/\text{hour}$  to  $2500\text{m}^3/\text{hour}$ .

Ballast water can be deballasted from a vessel and then, sometimes followed by reballasting the water back on board. According to Carlton et al. (1994), there are several reasons for this.

1. Weight compensation: A ship would have to deballast while taking on cargo, equipment, fuel, water, or personnel. A ship will either deballast in the port or harbor or deballast in calm seas while on its way into the port.
2. Port draft requirements: Ships may have a limit on how much ballast water they can carry while entering the port. This could have something to do with the depth of the port.
3. To compensate for density changes in the surrounding water: Due to buoyancy changes, a vessel moving from fresh to salt water may need to take on ballast. A vessel moving from salt to fresh water may need to discharge ballast.
4. Ballast water temperature control: A ship heading toward colder climates with freshwater ballast may change water to keep it from freezing.
5. Compensation for internal condensation: A ship heading towards warmer climates with colder ballast may change water due to condensation problems.



6. Compensation for fuel thickness: A ship with cold ballast water can experience fuel thickening if the ballast tanks are located next to the fuel tanks. The cold water is exchanged for warmer ambient water.
7. Increase in speed in calm waters: A ship may deballast water in calm seas in order to increase speed and decrease fuel consumption.
8. Discharge of polluted water: Water that is taken up in known polluted areas is deballasted on the open sea for cleaner water.
9. Discharge of sediments: Ships exchange water with high amounts of sediment for open ocean water.

There are certain situations in which ships avoid ballasting. Avoidance of ballasting up water in high sediment areas helps avoid sediment accumulation and additional weight, avoids removal costs, avoids shallow ballast tanks from filling up with sediment, and avoids the uptake of sulphate reducing bacteria which is the leading cause of microbially- induced ballast tank corrosion. Avoidance of ballasting up water that is believed to be polluted, can eliminate clean up costs in the tanks.

There are different types of ballast tanks depending on the kind of vessel. The establishment of segregated and dedicated ballast tanks came about through national and international efforts to reduce the discharge of oily ballast water into the ocean. Segregated ballast tanks are those tanks that carry only water and always have separate ballast piping. Dedicated tanks are cargo holds that have been unaltered and used exclusively for ballast (Carlton, 1985; Carlton et al., 1994). Permanent ballast may be solid ballast such as lead, pig iron, drilling mud, or concrete. Water can also be used but it is rarely changed which is called semi-permanent ballast. Further, the capacity of these

tanks can range from hundreds of gallons in sailing boats and fishing boats to tens of millions of gallons in commercial cargo carriers. Since there is no international standard on the unit of measurement, they are reported in capacities of metric tons, short tons, long tons, cubic meters, U.S. gallons, or Imperial gallons or barrels (Carlton et al., 1994). Table 1 refers to the specifications of ballast water capacities and distributions in six vessel types (Carlton et al., 1994). Units are in MT (metric tons). Total BW (ballast water) capacity shown in parentheses has been converted to U.S. gallons. Tanks and holds are indicated in brackets. Capacity shown in MT (metric tons)

Table 1: Specification of ballast water capacities and distributions in six vessel types

Vessel Type	Total B.W. Capacity	Tanks and Holds	Capacity (MT)
General cargo ship	4,200 (1,109,510)	Deep tank mid-ship aft	890
		Deep tank mid-ship forward	890
		Tunnel tanks	400
		Under-deck tank aft	20
		Under-deck tank forward	20
		[After peak tank, half-height deep tank, fore peak tank]	1980
Container ship	2,400 (634,000)	Deep tank forward	300
		Side tanks (in #1&2 holds)	1350
		[After peak tank, fore peak tank]	750
Bulk carrier	18,000 (4,755,060)	Topside tanks and holds	6000
		Combined bottom and side tanks	8000
		[After peak tank, fore peak tanks, #4 hold or deep tank]	4000
Ore Carrier	10,000 (2,641,700)	[After peak tank, fore peak tank, bottom tanks, side tanks]	10000
Tanker	20,500 (5,415,480)	"Clean ballast tanks" [side tanks]	14500
		[Half height] deep tank forward	3300
		[After peak tank, fore peak tank]	2700
Roll On	350 (92,460)	Deep tank forward [fore-peak tank, other tanks]	90
Roll Off Cargo			260

Normally, ships of various types carry ballast water proportional to their deadweight tonnage (DWT). Carlton, (1989) stated that a ship carries “up to 30 percent or between 25 and 35 percent of its dead weight tonnage.” Ballast capacity may be 25 percent dead weight tonnage on the average, 20 percent for short voyages, 30 percent dead weight tonnage for bad weather, and around 40 percent for severe conditions (Carlton et al., 1994; Locke et al., 1993).

Ships are said to be in ballast when they have ballast water on board and no cargo. Ships with ballast may have cargo and some ballast water on board. Ships on their ballast leg carry the most ballast water. Ships that are on their way into a port may or may not have released their ballast water prior to cargo loading, while ships leaving a port fully loaded with cargo can have very little ballast water. This condition can be reported as “no ballast on board”. Ballast water can remain on board because it is trapped in hold or tank spaces so that the pump cannot suction it out properly. This water is referred to as unpumpable water. According to marine biologists this unpumpable water may be capable of supporting a large variety of life. Basically, all ships have some form of ballast water on board at all times (Carlton et al. 1994).

According to Carlton et al. (1994), there are three categories of ballast water, two of which overlap:

#### Acknowledged Ballast:

Vessels in ballast as reported in official government records. The volumes that are actually on board and the volumes of water to be discharged are never recorded. Vessels with no cargo are recorded as in ballast,

regardless of the actual ballast condition. The last port of call data is available, but the last port of call is not specifically the source of the ballast water.

#### Unacknowledged Ballast:

Vessels with ballast water. This data is not reported to or by the government.

#### Cryptic Ballast:

Unacknowledged ballast, unpumpable ballast, reported no ballast on board when there is some ballast water on board, and ballast water on board vessels are not recorded by government records. At the present time there are no attempts to estimate the volume of foreign and domestic ballast water being transported by U.S. Navy military cargo and support vessels. There are also no attempts to estimate the amounts of ballast water and fouling organisms of semi-submersible exploratory drilling platforms and offshore drilling rigs being transported.

U.S. Customs and port records do not make it a habit to record the amounts of ballast water carried when vessels are in ballast, and do not normally record data on the presence of ballast water at all when the vessels are with ballast. Carlton suggests that the U.S. Customs Bureau ought to collect data on ballast and cargo conditions from arriving vessels, since this data is easily accessible (Carlton et al., 1994).

The age of ballast water has been defined as the length of time it is resident in the tank or hold. This can range from less than 24 hours to many months. Container ships and Roll on Roll off cargo ships travelling between coastal ports will re-ballast and de-

ballast water at different ports in less than a day. Some ships that have double bottom and peak tanks can take on permanent and semi-permanent ballast water. This water may take up residency from several months to year. There is not much known about the physical, chemical, or biological qualities of this old water (Carlton et al., 1994). According to a study performed by Williams et al., (1988), the longer the transit time the less likely living organisms will survive. According to Carlton (1985) "for many species transport is more likely to be successful over short distances than over long distances". Also, Carlton, (1985) found living organisms in 31-day old ballast water and copepods in 95 day-old ballast water. This does suggest that as long as old ballast water does not degrade below the ability to support life it can contain living organisms (Carlton et al., 1994).

Carlton's 1985 study discussed the physical and chemical characteristics of the environment of ballast water. Light or the absence of light may play a role in the survival of living organisms being transported from one place to another. Since there is no light in ballast tanks this can effect stages of certain organisms. Studies have been performed on calanoid copepods. It was found that calanoids reared under mostly lighted conditions produced eggs that hatched immediately. Calanoids that were reared under mostly dark conditions, produced "resting" eggs or eggs that did not hatch immediately. If survival conditions are good, dormant egg producing copepods are more likely to produce resting eggs, which can be dispersed in the ballast tanks. The absence of light may also be crucial to predators that rely on seeing their prey. Studies have shown that fish and their larvae can obtain food in the dark (Carlton, 1985).

The temperature within the ballast water tanks and holds might also be critical in the transport of living organisms. The ballast-tank temperatures vary. They may increase, decrease, remain constant, or become cyclic. According to several studies, temperature controls food satiation, fertilization, and growth of different types of organisms. The existence of seasonally-dependent temperature tolerances and egg-production rates by a number of species of copepods has important implications for ballast water biology, suggesting that populations of the same species ballasted at different times will have different response regimes to changing temperatures during transport (Carlton, 1985; 1987).

Food availability is also critical in the transport of living organisms via ship ballast water. According to Carlton, (1985) food availability can be visualized as fixed and/or declining resource. The continual production of bacteria is an exception because it can support organisms, such as copepods, for several months. "There are certain larval and adult invertebrates that have evolved a number of mechanisms to compensate naturally for low food concentration conditions. These starvation and post-starvation recovery adaptations may lead a selective advantage to some taxa (such as *Psuedocalanus* and *Calanus*) for survival in ballast tanks" (Carlton, 1985). There are other variables to be considered as well. These include oxygen content, water quality, which refers to the amounts of organic and inorganic pollutants within the water, salinity, pH, and sediment load. Salinity may remain stable throughout a given voyage but oxygen content and temperature can vary a great deal (Carlton, 1985).

It is obvious that living organisms can be transported from one place to another through the uptake and release of ship ballast water. Figure 1 is a schematic showing

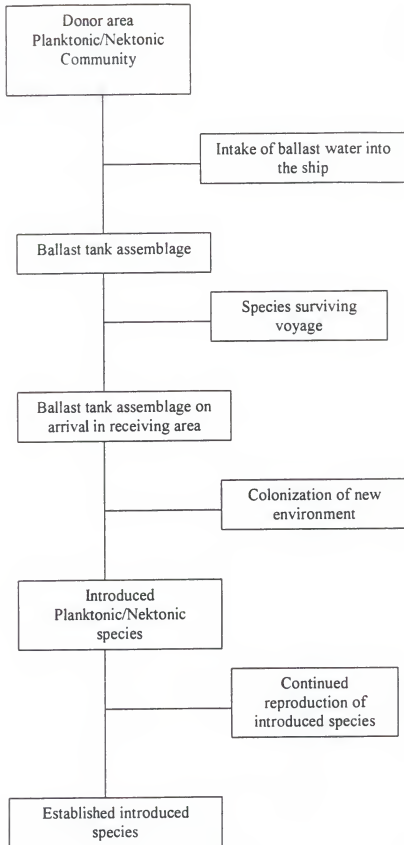


Figure 1: Species Introduction (modified after Carlton, 1985)

the sequence of events that most likely occur during uptake, transport and release of living organisms (modified after Carlton, 1985).

### Effect of Ballast Water on Movement of Exotic Species

According to Carlton et al., (1994) there are many kinds of species of living organisms that can be transported from place to place through ballast water. The range of organisms is vast. A list of organisms that have the potential to be ballasted into ships include the following:

#### Haloplanktonic Organisms:

These are organisms that spend most or all of their lives in the water. In coastal and marine systems these include: Phytoplankton (diatoms, dinoflagellates, blue-green algae, nannoplankton, autotrophic picoplankton, and other groups) and Zooplankton (comb jellies, jellyfish, hydrozoans (siphonophores), polychaete worms, rotifers, gastrotrichs, planktonic gastropods (snails: the pteropods and heteropods), copepods, hyperiid amphipods, isopods, mysids, ostracods, cladocerans, pelagic shrimps, krill (euphausiids), arrow worms (chaetognaths), pelagic tunicates (including salps, doliolids, and larvaceans)), and fish.

Neustonic organisms, those that occur at or near the air/sea interface, can be ballastable if carried by turbulence or down-welling to the depths of the ballast intakes. These organisms include larvae and juveniles of the by-the-wind-sailor *Veilella*, the blue button *Porpita*, nauplii and cyprids of the barnacle *Lepas*, and the sea strider *Halobates*. An often over-looked role of ballast water is its ability to move open water species between ocean



basins. In high seas, oceanic organisms such as radiolarians, silicoflagellates, globigerinid foraminiferans, copepods, and chaetognaths, otherwise restricted by major ocean barriers and temperature boundaries, can easily be transported and released between ocean basins.

#### Meroplanktonic Organisms:

These are organisms that spend a portion of their lives in the water column. In coastal and marine waters these include: Phytoplankton (the dispersal of benthic plants) and Zooplankton (the larvae of many invertebrates, including sponges, sea anemones, corals, hydroids, mollusks (snails (including sea slugs, or nudibranchs), chitons, and mussels, clams, oysters, and scallops), crustaceans (barnacles, shrimp, lobsters, crabs, hermit crabs), nemerteans (ribbon worms), sipunculans, polychaete worms, bryozoans, phoronids, echinoderms (sea stars, brittle stars, sea urchins, sea cucumbers), hemichordates, tunicates (sea squirts), and the larvae of fish. Oceanic teleplanic (long-distance dispersing) meroplankton (larvae) can also be transported via ship ballast water between oceans and ocean basins.

#### Demersal Organisms:

These are organisms that migrate vertically up into the water, usually at night. Organisms that live mainly in shallow water rise up off the bottom and become common in the water at night. These organisms include: a variety of small crustaceans (gammarid amphipods, isopods, mysids, cumaceans, crangonid and other shrimp, and benthic harpacticoid

copepods), fish species, and polychaete worms. When these types of organisms are present in the ballast water, the ship probably ballasted part of its water at night.

#### Tychoplanktonic Organisms:

These are bottom organisms that can get swept up into the water column (tidal currents, waves, ships' propellers) and remain buoyant in the water for varying lengths of time. Examples of these organisms include: forams, flatworms, polychaetes, crustaceans (copepods, amphipods, isopods, and tanaids), hydroids, benthic copepods, insect larvae, mites, and nematodes.

#### Benthic Organisms in Sediments:

These are organisms that can be brought into a ship with bottom sediments. These include all of the groups listed above, as well as leeches, oligochaete worms, insect larvae, and adult insects.

#### Floating Detached Biota:

A large variety of floating, detached organisms can be drawn into a ship. These organisms include: sea-weeds (algae), sea-grasses (eelgrass, sargassum, turtle grass), and marsh plants, and epiphytic organisms on the blades of floating plants. These include: spirorbid tubeworms, bryozoans, seasquirts, sponges, mollusks, and crustaceans.

#### "Migratory" Organisms:

"Migratory" organisms often undergo nocturnal excursions (migrations).

An example would be the wood-boring gribble Limnoria, a tiny isopod

crustacean. This organism migrates by swimming between wood habitats and can be taken up into ballast tanks through gribble-infested wood (Carlton, 1985).

#### Fish and Shellfish Diseases, Pathogens, and Parasites:

Marine diseases, pathogens, and parasites can also be transported by ballast water along with mariculture and aquaculture diseases.

When a species has spread beyond its traditional range it is considered alien or exotic (Bederman, 1991). "Homogenization of the planet is a process both inevitable and inexorable, as species after species casts itself abroad" is the description of the phenomenon of the spread of exotic species (Bederman, 1991). According to Carlton (1985), biological invasions occur through two processes; range expansions and introductions. Range expansions occur when the dispersal of an exotic species is done through natural mechanisms. Introductions consist of transportation by humans, usually across natural barriers, into a region where the species did not exist. Carlton (1989) and Macleod (1995) refer to this as "human-mediated dispersal". Marine communities are composed of indigenous species (those species that are naturally found) whose length of existence in the community is generally not known. Relatively recent arrivals are considered range expansions and/or introductions. Cryptogenic species are those species not assignable to either one of these categories (Carlton, 1987; 1989; Macleod, 1995). According to Leppakoski (1991) there are four successive stages of invasions: arrival, establishment, spread, and persistence. The invading specie's ultimate success may be evaluated through persistence time. The longer they can survive in the region they were released the better their chances of long term survival (Leppakoski, 1991; Moyle, 1991).

According to Sheehan (1995), the success of biological invasions has been attributed to several factors. These factors include:

1. Absence of natural enemies,
2. High dispersal capacity,
3. Early breeding, high fertility, rapid development, and small size,
4. Vacant niches.

A study performed by Herbold and Moyle (1986), tends to refute the idea of vacant niches. There are many definitions of the term "niche" and according to the study, very few of them apply to "vacant" niches without contradiction. If they do apply, the resulting vacant niches are hard to identify. An introduced species rearranges the community rather than slips into an empty slot. Most successful introductions are made into habitats that are already disturbed. If there are introductions made into undisturbed habitats, the native species usually will be displaced through competition (Herbold and Moyle, 1986; Moyle, 1991; Carlton and Geller, 1993; Macleod, 1995). It is hard to determine the impact of an alien species in a particular ecosystem, since, the effect will often depend on its biological attributes. These attributes include survivability in unfavorable conditions, adaptability to new environments, reproductive capability and the ability to disperse rapidly. Negative implications of introduced species include habitat destruction, competition or predation, hybridization, and the possibility that alien parasites and diseases may also be introduced. Hybridization may lead to loss of genetic diversity which can also lead to extinction of certain species (Sheehan, 1995). New diseases may further aggravate the condition of estuarine populations, which are already

stressed due to dredging, pollution, and general water quality conditions (Moyle, 1991; Suchanek, 1994).

According to Cognetti and Curini-Galletti (1993), the marine environment is more stable than the terrestrial. As the depth of the marine environment increases the environment becomes more stable. Carlton (1989) suggests that shallow water areas are at risk more than deep water areas. The more constant the physico-chemical parameters, the more stable the communities, which tends to be characterized by high specific diversity and high levels of genetic variability within the population. Specific diversity decreases as the intensity of disturbance increases, which operates through a gradient of physiological stress. This leads to a decrease in the number of species and an increase in the number of individuals of species able to withstand the increasing environmental problems. According to Cognetti and Curini-Galletti (1993), "opportunistic" species are characterized by populations with high reproductive rates and low genetic variability. This is due to selection favoring very few genetic characteristics that are able to withstand a wide range of variation of environmental parameters. These situations are found most frequently along coasts that are close to urban or industrial areas where there is little water depuration. In some of these areas, oil and urban refuse is polluting the environment (Cognetti and Curini-Galletti, 1993; Omori et al. 1994).

Many marine organisms represent "instantaneous" alterations over the past hundreds of years, rather than over thousands of millions of years of dispersal and isolation occurrences. Human-mediated dispersal mechanisms of marine organisms, combined with other evidence derived from biogeography, genetics, evolutionary ecology, systematics, distributional ecology, history, archeology, and paleontology, may

be leading scientists to consider, not how a species is introduced, but how it is known that a species is native to an area. There are many areas that have been altered by system-specific events. Some of these areas include the bays of the Pacific coast of North America, the coral reef flats of the Indo-Pacific and Hawaii, the rocky shores of New England, and the fouling communities and mudflats all over the world. It is believed that these are the areas that have been altered by introduced species, and whose natural conservation is of greatest concern due to these invasions. The rocky shores that have high impact waves, open coast sub-tidal waters, and kelp beds of the Pacific coast are examples of systems that are believed to have not been altered by introduced species, but have been to some degree by other human-mediated disturbances (Carlton, 1989; Moyle, 1991; Carlton and Geller, 1993).

According to The International Maritime Organization, if fresh water (FW), brackish water (BW), and fully saline or salt water (SW) are considered, an indication of probability that aquatic organisms and pathogens would survive after transfer from one place to another can be discussed (see table 2 below).

Table 2: Probability of organism transport in different salinities (Anon, 1993b).

Discharged Waters	FW	BW	SW
Receiving Waters			
FW	HIGH	MED	LOW
BW	MED	HIGH	HIGH
SW	LOW	HIGH	HIGH

Brackish and salt water have the greater potential for successfully transplanting pathogens and aquatic organisms from one place to another (Anon, 1993b). Good

examples of this are the Baltic and Black Seas. The surfaces of both seas are brackish between 8,000 to 18,000 feet. This allows for successful introductions from higher salinity bodies of water such as the East Atlantic river mouths and the North Sea (Leppakoski, 1991).

Many studies carried out in several countries that have shown many species of bacteria, plants, and animals can survive in viable forms in ballast water and then be transported to another destination where they have survived and prospered (Anon., 1993b). According to a study performed by Hutchings (1992), sightings of three fish species, known only to South-East Asia, reported around Australian ports in the 1970's. Each one of these species is associated with ballast water discharging. The yellow-fin goby (*Acanthogobius flavimanus*) was first found in Australia in 1971 and is now established in Sydney Harbor, Botany Bay, Hawkesbury River, and Port Kembla. This species was also introduced and now is established in San Francisco Harbor through ballast water discharge. The striped goby (*Tridetiger trigonocephalus*) has become established since 1972 in Sydney Harbor and Port Kembla in New South Wales. It has become established also in the harbors of San Francisco and Los Angeles. The third species, introduced around 1982, is the Japanese sea bass (*Lateolabrus japonicus*), which was sighted in Pittwater and Botany Bay in New South Wales. These three species may become a problem because they are carnivores and can eliminate smaller native goby species and juveniles of other fish (Hutchings, 1992).

In the Great Lakes region, a round bug-eyed goby was introduced in the 1980's in ballast water chambers from the Black and Caspian Seas. It now threatens to disrupt the Great Lakes' ecosystem and it potentially raises the risk of people being exposed to

cancer-causing PCBs. Since its introduction, the round goby has become a dominant species along wharfs and docks in Cleveland. It eats off the rocky bottom, which is home to many other native species such as the sculpin and darter. The goby can grow to nine inches and can drive off other fish and eat their eggs or young. It is basically competing with native fish for food and living space. Europeans consider this fish edible but the United States government disagrees and warns against eating it due to the potential of PCB contamination. The zebra mussel, also introduced to the Great Lakes region through ballast water release, filters about a quart of water a day which allows for the absorption of the cancer-causing PCBs. The gobies eat the zebra mussels, which in turn are eaten by the walleye sport fish. The walleye are then consumed by people which in turn puts them at risk of PCB poisoning. This can become a major health problem for the American people (Anon., 1997a).

The American jackknife clam, *Ensis directus*, made its way to the European coast and in the German Bight around June of 1979. It is assumed that the clam was transported in its larval stage by a cargo ship's ballast water. Since its discovery, the clam has spread rapidly through the North Sea in sub-tidal and inter-tidal areas. Dense populations of the clam were found along the German coast within a few years after its discovery. Other places where the clam has been detected are the North and East Danish coasts and the Belgian coast. There were no other reported sightings until June 8, 1991 when the clam was found in muddy, fine sand along the French coast of the North Sea at a water depth of around ten meters. In February of 1992, it occurred in French coastal waters and extended to the western harbor of Dunkerque. It is thought that the northerly



winds transported the larvae in the opposite direction to the northeasterly residual current (Lucsak, et al.1993).

According to Gosliner (1995), of the 217 species of opisthobranch gastropods (mollusks) reported from the Pacific coast of North America, only six are likely to have been introduced through human activities. Specimens of an unidentified opisthobranch from the southern part of San Francisco Bay were discovered July, 1993. The specimens were later identified as *Philine auriformis*. This organism is known to inhabit waters of New Zealand and possibly southern Australia. Another population of opisthobranchs were discovered in April, 1994 in the inter-tidal mudflats of Bodega Harbor, located around 120 km north of the entrance to San Francisco Bay. Scientists are concerned about the impact these organisms can have on the soft-bottom benthic communities. In New Zealand waters, *Philine auriformis* feeds mainly on protobranch bivalves of the genus *Nucula*. In San Francisco Bay, the mollusks were most probably introduced by being taken up into cargo ship ballast chambers in larval form and discharged into the Bay. *P. auriformis* would mainly feed on another introduced mollusk, *Gemma gemma* in San Francisco Bay, while in Bodega Harbor, it would feed on native *transennella* clams. Therefore, *P. auriformis* is considered an opportunistic predator on small benthic bivalves. The species of *transennella* are the primary source of food for wading birds. If the native bivalves are depleted by *P. auriformis*, this can have a major adverse competitive effect on native shorebird populations (Gosliner, 1995).

According to Marsden (1993), at least 87 non-indigenous species have been released or introduced into the Great Lakes region. Several of the species include the sea lamprey, alewife, and smelt, which have contributed to huge alterations to the Great

Lakes fish community. The blue pike is now extinct and the kiyi and shortnose cisco have been severely diminished due to competition with the smelt. The lake trout and whitefish populations have almost disappeared from several lakes because of the lamprey predation along with the effects of over-fishing. The native populations of chubs, burbot, emerald shiners, and sculpins have been displaced by introduced species. The lake trout have been replaced by the Pacific salmon, while the native forage fish populations have mainly been replaced by alewife and smelt. The zebra mussels (discussed below) have directly affected the human community by fouling boat hulls, water treatment plants, and littering recreational beaches with piles of broken shells (Marsden, 1993).

Jellyfish that Iranians say were carried by oil tankers from the Black to the Caspian Sea threaten to ruin Iran's \$34 billion caviar industry. Officials say that the "foreign" jellyfish are decimating the Caspian's sturgeon population, which is the source of the Beluga caviar (Newman, 1998).

An exotic freshwater bivalve, *Preissen polymorpha*, the zebra mussel was released into Lake St. Clair/Detroit River in the Great Lakes in 1985. It was released through the discharge of cargo ship ballast water, which was carried from a freshwater European port. The mussel's larval stage has allowed it to spread rapidly downstream throughout the shallow near shore of Lake Erie. The mussels now extend into Western parts of Lake Michigan, Green Bay, Wisconsin, Cornwall, and several other locations on the St. Lawrence River. At one time, this species was restricted to the Caspian Sea and Ural River in Asia. It spread throughout the European freshwaters in the 19<sup>th</sup> century and is now in the western parts of the Soviet Union and southern Turkey. Once the larvae are settled in low-flow areas, they attach to hard surfaces by proteinaceous threads or

"byssal" threads. They then grow in size and accumulate in number, which can either reduce or block water flow. The mussels secrete byssal threads from the base of the foot and form a tough hold fast which high flow velocities cannot dislodge (McMahon and Tsou, 1990).

In hydroelectric stations in Europe, zebra mussels have fouled piping, walls of turbine-bearing chambers, cooling pipes of the mercury valves of generator ionic exciters, and intake trash racks and gates. In steam-electric power plants, the mussels foul piping, small diameter components, intake structures, trash racks, and screening. The power plants and municipal water treatment plants on Lake Erie have already reported zebra mussel infestations (McMahon and Tsou, 1990; Bolch and Hallegraeff, 1993). Zebra mussel fouling problems are likely to be most severe and difficult to manage in service water systems because portions are difficult to access for mussel removal. The macrofouling of the zebra mussel now costs the United States power industry over one billion dollars per year (McMahon and Tsou, 1990). Over a period of 10 years an estimated cost of controlling the zebra mussel can reach as high as 5 billion dollars (Bolch and Hallegraeff, 1993). As the zebra mussels increase in number, so will the costs to repair damages done by the mussel in the future (McMahon and Tsou, 1990).

Unfortunately, once the zebra mussel was introduced and became established, it has been very hard to get rid of (Marsden, 1993). There are several options that are being looked at in controlling the zebra mussel. The use of a number of biocides has been tested against the zebra mussel over spawning periods. Chlorination has been widely used in Europe with some success. Ozonation is also another option being considered because it prevents mussel settlement and growth in municipal water utility piping receiving water

from Lake Erie. Laboratory experiments also show that the non-oxidizing biocides poly[oxyethylene(dimethyliminio)ethylene-(dimethyliminio)ethylene dichloride] and 2-(thiocyanomethylthio)benzothiazole killed the zebra mussels at low concentrations. Back washing or recirculation of heated water into intake structures can reduce zebra mussel accumulations. Antifouling, biocide surface coatings such as copper and nontoxic silicone coatings prevent successful accumulations of zebra mussels. The use of electric pulses passed through metallic intake structures kill juvenile mussels. In some systems annual cleaning may be of some benefit in removing adult mussels or would prevent build up in piping. The basic goal is to keep the mussels from settling or reaching sizes or densities that could foul tubing or piping (McMahon and Tsou, 1990).

According to another study, the zebra mussel was found at Harvey Lock across the Mississippi River close to New Orleans. They were found on the empty/filling culvert valve at Harvey Lock, which is operated and maintained by the U.S. Army Corps of Engineers. The discovery of the zebra mussel in the New Orleans area marks the southernmost sightings of the organism in the United States. Harvey Lock is only 98.5 river miles from the mouth of the Mississippi River. The zebra mussel was also discovered at the Louisiana Hydroelectric Company power plant several months earlier in fire control water strainers (Anon., 1993a).

The San Francisco Bay area, back in 1862, suffered a great flood, which turned the bay into fresh water for a week. This forced the estuarine mixing region out to sea beyond the Golden Gate Bridge and made this region susceptible to foreign invasions of other organisms. The completion of the transcontinental railroad in 1867 allowed the transport of live oysters from the Eastern seaboard to San Francisco, which introduced

other organisms living in or on the oysters into the Bay area. In 1985, a new invader was discovered in the upper arm of San Francisco Bay and was thought to have been released through ballast water. This invader is the Asian clam *Potamocorbula amurensis*, which lives in a wide range of salinities. It can seriously disrupt the food chain of San Francisco Bay by eating diatoms and larvae of different native crustaceans. Another invader, found in 1990, and thought to have been released in ballast water, might come to the rescue. The European green crab *Carcinus maenas* is very fond of mussels and sea urchins. It is even known to bite people. This particular crab is related to the Eastern blue crab of the United States and is a veteran traveler in cargo ship ballast water. It has reached as far away as Australia and South Africa. The question is, if the green crabs eat all of the clams what will they start eating next (Hedgpeth, 1993)? In Morehead City, North Carolina, scientists are concerned about the potential effect of foreign ballast water on aquatic life in their area. Scientists are trying to find out what is being transported into the ports. Inspectors are trying to examine cargo coming into the state ports at Morehead City and Wilmington for pests and other visible potential problems. North Carolina is known for its native blue crab population and officials are concerned about an invasion of the Japanese green crabs. The Japanese green crab has the potential to wipe out the blue crab population if it is accidentally introduced to the area (Anon., 1997b).

According to Anderson (1993), at least fourteen organisms were introduced from ballast water and become established in parts of the local marine life in Australia. These organisms range from dinoflagellates, mollusks, crustaceans, and even two fish species. There could be many other introduced species in the Australian area that no one knows about (Anderson, 1992). There was a report on a starfish that was a voracious eater and

had the capacity to inhabit large areas. It was, and is, causing damage to endemic and commercially valuable species. The starfish, *Asterias amurensis*, is considered a threat to Australia's shellfish industry. Every time a vessel takes in water with starfish eggs and larvae, it has the potential to spread the starfish to wherever the saltwater is discharged (Anderson, 1993). Over the past several years, shellfisheries in Tasmania have been closed due to several breakouts of toxic dinoflagellates. These toxic dinoflagellates were released through ballast water exchange (Anderson, 1992).

Cargo ship ballast water has been proven to be responsible for the translocation and establishment of a wide range of marine organisms, including several toxic dinoflagellate species and other harmful exotic creatures. Many of these introductions have resulted in severe ecological economic problems (Rigby et al., 1995; Bolch and Hallegraeff, 1993). A survey of cargo ships entering Australian ports showed that 6% carried resting cyst stages of the toxic dinoflagellates *Alexandrium catenella*, *Alexandrium tamarense*, and *Gymnodinium catenatum*. These species have the capability to contaminate shellfish with paralytic shellfish poisons (Bolch and Hallegraeff, 1993; Hallegraeff and Bolch, 1992; Hallegraeff and Bolch, 1991; Scholin and Anderson, 1994; Scholin et al., 1994). Paralytic shellfish poisoning can kill humans and occasionally kills fish, birds, and other mammals that eat shellfish that are contaminated (Bolch and Hallegraeff, 1993; Hallegraeff and Bolch, 1992; Hallegraeff and Bolch, 1991). There is an apparent global increase in the frequency, intensity, and geographic distribution of paralytic shellfish poisoning (PSP) (Hallegraeff, 1995; Burkholder et al., 1992, Hallegraeff and Bolch, 1991 and 1992). There is a 15% mortality to humans who consume contaminated shellfish. Until 1970, poisoning records were confined to

temperate waters of Europe, North America, and Japan. However, by 1990 paralytic shellfish poisoning was confirmed throughout the Southern Hemisphere including South Africa, Australia, New Zealand, India, Thailand, Brunei, Sabah, the Philippines, and Papua New Guinea. The increase of apparent PSP outbreaks is due to scientific awareness caused by development of aquaculture industries and the stimulation of dinoflagellate blooms by increased coastal eutrophication (Hallegraeff, 1995; Hallegraeff and Bolch, 1991 and 1992; Bolch and Hallegraeff, 1993).

There has been work done to explore the possibilities of physical and chemical treatment options for ships' ballast water using toxic dinoflagellate cysts as model organisms. Treatment mechanisms that kill resistant dinoflagellate cysts are more than likely as effective in killing other marine organisms such as larval zooplankton, copepod eggs, and seaweed spores. However, bacterial spores and viral particles may not be affected and another alternative might be used. In laboratory studies, a short 30-90 second heat treatment might be the solution for the global ballast water problem, and it is environmentally friendly, although not economical. The chemical tests showed that dinoflagellate- resting cysts had a high chemical resistance as compared to the fragile motile plankton cells. The microbiocide Kathon WT 1.5% was completely ineffective after 30 times the recommended dose. High concentrations of free chlorine and hydrogen peroxide were effective in killing dinoflagellate cysts. Chlorine-based biocides are commonly used to reduce levels of bacteria, viruses, algae, and fungi in human water supplies, swimming pools, sewage-effluent, and industrial water systems. Free chlorine levels as high as 500ppm were necessary to kill *G. catenatum* dinoflagellate cysts. Due to the concentration, this is a very expensive option and not environmentally friendly. It

would take 400t of 12.5% sodium hypochlorite to treat a 50000t ballast tank at a cost of \$200,000. Hydrogen peroxide is more environmentally friendly but costs even more than the free chlorine treatment. To treat a 50000t ballast tank would require 1000t of industrial 50% hydrogen peroxide solution at a cost of \$2,000,000 (Bolch and Hallegraeff, 1993; Rigby et al., 1993; Rigby et al., 1995; Hallegraeff and Bolch, 1991; and 1992). The present studies indicate that the best method in preventing the spread of toxic dinoflagellate cysts via ship ballast water would be to avoid taking on ballast water during dinoflagellate blooms in the water column of ports all over the world (Hallegraeff and Bolch, 1992).

Not just organisms that damage marine animal and plant life are released, but bacterial organisms that hurt humans are found as well. A strain of *Vibrio cholerae* has been found in the ballast water of three ships that visited ports on the United States Eastern seaboard after stopping in Latin America. The organism was then discovered in the ballast water of two more ships (Anderson, 1992). According to Epstein, (1993), up until the 19<sup>th</sup> century, *V. cholera* was confined to Asia, and almost exclusively to the Ganges and Brahmaputra River basins in India. Now, correlated with faster sea transport, *V. cholerae* has spread even faster after 1895. Outbreaks have even accompanied air transport. Drifts and shifts in types, strains, and virulence of the bacteria can be related to the environment and pressures on host and agent, and the efficiency of transmission (Epstein, 1993). In January of 1991, epidemic cholera was reported in Peru and was spreading quickly through Latin America and into Mexico. To show how fast this organism was spreading, it was isolated from five oysters and one mackerel fish from Mobile Bay, Alabama in July and September of 1991 and June of 1992. After studying



the biochemical and serological characteristics, the isolates showed a resemblance to the Latin strain of *Vibrio cholerae*, *Vibrio cholerae* 01 (McCarthy and Khambaty, 1994). The survivability of the toxic *V. cholerae* 01 aboard cargo ships ballast water is probably enhanced by its attachment to particles (McCarthy and Miller, 1994). Studies have shown that *V. cholerae* attaches to algae and other chitinous fauna such as copepods, crabs, and shrimp. This apparently helps improve the survivability of *V. cholerae* as it is transferred from one place to another in cargo ship ballast water. *Clostridium botulinum* C was found in a Norwegian vessel that docked in Queensland after docking in Singapore. However, only 1 of 281 ballast tanks that were sampled contained the organism (Anderson, 1992).

#### Methods and Regulations for Ballast Water

There are many methods considered in the treatment of cargo ship ballast water. These methods (taken from the International Maritime Organization) include:

- treatment by chemicals and biocides;
- heat treatment;
- oxygen deprivation;
- tank coatings;
- filters; and
- ultraviolet light disinfection.

In determining the appropriate strategies for ballast water and sediment discharge, The International Maritime Organization (I.M.O.) has determined that the following criteria be considered for ballast water discharge procedures:

- operational practicability;

- effectiveness;
- seafarer and ship safety;
- environmental acceptability;
- water and sediment control;
- monitoring; and
- cost effectiveness.

According to the I.M.O., there are several approaches that might be effective in controlling the incidence and introduction of aquatic organisms and pathogens. These include:

- the non-release of ballast water;
- ballast water exchange and sediment removal at sea or in areas designated as acceptable for the purpose by the State Port Authority;
- ballast water management practices aimed at or preventing or minimizing the uptake of contaminated water or sediment in ballasting and deballasting operations; and
- discharge of ballast water into shore-based facilities for treatment or controlled disposal.

The means of preventing the introduction of unwanted aquatic organisms and pathogens from ships' ballast water and sediments is to avoid the discharge of ballast water, wherever possible. The exchange of ballast water in deep ocean areas or open seas presently gives a means of limiting the probability that fresh water or coastal marine organisms will be transferred in ballast water (Anon., 1993b). Ships' crews should be made aware of the ecological and health hazards posed by the careless loading and

discharging of ballast water. Ships' crews also need to understand the importance of maintaining tanks and equipment, such as anchors, cables, and pipes, free from sediment (Anon, 1993b; Carlton, 1985; 1987; 1989; Carlton et al., 1994; Hallegraeff and Bolch, 1991 and 1992).

In February 1990, the Australian Quarantine Inspection Service (AQIS) introduced voluntary guidelines called "Controls on the Discharge of Ballast Water and Sediment from Ships Entering Australia from Overseas". These guidelines provided vessels with a number of options aimed at reducing the possible introduction and spread of exotic and harmful organisms. It also included random sampling of the contents of ballast tanks to establish a profile of the organisms found in vessels entering Australian ports. The options, provided by the AQIS, include:

- certification from an overseas government indicating absence of toxic dinoflagellates at the time of taking on ballast water,
- evidence from reballasting at sea en route to Australia, along with a promise not to release ballast water while in Australian waters, coupled with a "Compliance Agreement" to maintain ballast contents in a clean condition,
- and implementation at an approved treatment process to eliminate possible harmful organisms.

In July of 1991, the Marine Environment Protection Committee (MEPC) recommended that the International Maritime Organization (IMO) implement international controls on the movement and discharge of ballast water around the world. In the meeting in London, England, chaired by Australia, a unanimous agreement implemented voluntary guidelines that suggested a range of options. These options were

aimed at controlling the pick up and transport of exotic organisms. The guidelines include:

1. Reballasting at sea and sediment removal (exchanging ballast water in mid ocean to eliminate organisms picked up in port).
2. Non-release of ballast water in ports (not always possible with empty bulk carriers).
3. Ballast water management practices to minimize the transport and possible release of exotic organisms.
4. Onshore treatment facilities for the safe disposal of ballast water and sediment.
5. Treatment of the ballast water, in an approved manner, to eliminate exotic organisms prior to discharge (no known treatment available at this stage).

The ratification of the guidelines by the IMO in November of 1991 saw them introduced by all 132 member countries, which implemented them in all the world's major and smaller shipping nations. Compliance with the guidelines are reported to IMO at regular intervals to make sure they are fully implemented (Anon., 1992; Anon, 1993b).

The IMO adopted Resolution A. 774(18) "Guidelines for preventing the introduction of unwanted aquatic organisms and pathogens from ships' ballast water and sediment discharges", in November of 1993. These guidelines stressed that international cooperative measures were essential because the uncontrolled discharge of ballast water and sediments "has important global implications" (Sheehan, 1995). These guidelines are similar to those adopted by AQIS. They differ from the United States Non-

indigenous Aquatic Nuisance Prevention and Control Act of 1990, in that they are more flexible (Bederman, 1991).

Canada and the U.S.A. have also introduced similar controls for vessels entering the Great Lakes and are moving to introduce these guidelines for all of their ports (Anon., 1992). The Canadian Coastguard issued a set of voluntary guidelines in 1989, and jointly with the U.S. Coastguard in 1991. The Great Lakes Ballast Water Control Guidelines (GLBWCG) was implemented and intended to limit unplanned introductions of nonnative species into the Great Lakes. The GLBWCG requires ships destined for the St. Lawrence Seaway and upper St. Lawrence River to replace all ballast taken on in fresh or coastal marine waters with mid ocean water (Locke et al., 1993). In November of 1990, legislation was enacted by the U.S., that required mandatory compliance by the shipping industry with ballast exchange (Public Law 101-646, Stat. 4761, Non-indigenous Aquatic Nuisance Prevention and Control Act). The Act requires that within 2 years, regulations for ballast water exchange is to be issued. Failure to comply with the regulations would be a class C felony and can result in a fine of \$25,000 (Sheehan, 1995; Bederman, 1991).

In the United Kingdom, the Scottish River Purification Authorities (RPAs) comprising seven river purification boards and three island councils, have the duty under the Rivers (Prevention of Pollution)(Scotland) Act 1951 to ensure the cleanliness of the waters in their areas. The Control of Pollution Act of 1974 (COPA) has the power to enable the RPAs to fulfill this duty, which requires dischargers to apply for consent to discharge. Unfortunately, the COPA is quite definite that no consent is required for discharges from ships. However, it is an offense to cause or knowingly permit the discharge into controlled waters of a poisonous, noxious, or polluting substance. This

does apply to discharges from vessels and the sampling also applies to vessels. This makes it legal for the RPA to sample ballast waters to determine whether a breach of the COPA has occurred. The ships, however, could not be prevented from discharging. Scotland needs to have stiffer laws and penalties for uncontrolled cargo ship ballast water discharging (Macleod, 1995).

### Review of Work from Other Institutions

#### Ireland

In February of 1995, Jonathan Sheehan wrote his Master's thesis in cargo ship ballast water called, "Ships' ballast water as a vector for the introduction of non-indigenous species: implications for Ireland", at The University of Dublin, Trinity College. Sheehan (1995) assessed the risk of ballast water introductions to Irish waters by:

1. Observing the shipping practices of twelve main commercial ports- Belfast, Larne, Warren Point, Londonderry, Dublin, Cork, Waterford, Limerick, Foynes, New Ross, Drogheda, and Galway.
2. Looking at the imports, exports, and number of ships passing through the twelve ports (Table 3).
3. Examining the ports of Dublin, Cork, Waterford, and Limerick for determination of the origin of the ballast water, and donor regions, dumped into Irish waters (Tables 4,5,6, and 7).

Sheehan stated that Dublin, Limerick, and Waterford did not appear to be at risk from direct introductions of non-indigenous marine organisms from non-European ports because;

- They do not have a history of introductions.
- Nearly all of the ships visiting the ports were from coastal, cross-channel, or European ports.
- The quantity of ballast water discharged appeared insufficient to facilitate a successful introduction.
- With the exception of Waterford port, ships that traveled from non-European ports had insufficient repetition for an introduction to occur.

Cork Harbor, however, was at risk from ballast water introductions. According to Sheehan, this was due to;

- The port's long history of human-mediated introductions.
- A significant number of ships, both domestic and international, discharging ballast water in the port.
- A repetition of ship arrivals from both coastal and non-European ports.

Table 3: The twelve main Irish ports used in study (n.s. = not stated)

Port	Imports (tonnes)	Exports (tonnes)	No. of Vessels
Belfast	8142000	2550000	n.s.
Dublin*	5000000	3500000	3721
Cork	4111800	2618814	2600
Limerick	5808926	1116039	483
Larne	2328014	2006166	5583
Warrenpoint	1428618	835280	1183
Waterford**	1005061	695312	682
Foynes	1122638	157832	284
New Ross	990244	65312	551
Drogheda	538285	232604	508
Londonderry	767712	20818	n.s.
Galway	391687	5254	n.s.

\*Approximate throughput figures for 1993

\*\*Livestock exports not included

Table 4: Number of vessels visiting Dublin Port 'in ballast' in 1993 and volume of ballast water discharged by commodity and last port of call (LPC) of vessels

Commodity	Number of Vessels				
	Coastal	Cross-Channel	EU	World	Open Sea
BS	69	30	16	2	0
BB	1	6	0	0	42
BL	1	16	0	0	0
LO	37	6	3	0	0
RO	2	1	0	0	0
Total	110	59	19	2	42
Exports (tonnes)	268218	142841	97948	13066	92817
Ballast water (tonnes)	80465	42852	29384	3920	27845

(BS= Bulk Solids; BB=Break Bulk; BL= Bulk Liquids; LO= Load On; RO= Roll On)

Table 5: Number of vessels visiting Cork Harbor 'in ballast' in 1993 by commodity and LPC of vessels

Commodity	Number of Vessels				
	Coastal	Cross-Channel	EU	Other European	World
Oil etc.	181	92	12	1	0
Chemicals	95	53	3	0	0
Steel	62	22	9	0	0
Wood	41	35	1	0	0
Livestock	0	1	2	2	45
Foodstuffs	9	4	7	2	2
Total	388	207	34	5	47



Table 6: Number of vessels visiting Limerick Port 'in ballast' in 1993 and volume of ballast water discharged by commodity and LPC of vessels

Commodity	Coastal	Number of Vessels			
		Cross-Channel	EU	Other European	World
Alumina	27	42	35	4	2
Moss peat	4	0	2	0	0
Wood pulp	4	0	0	0	0
Total	35	42	37	4	2
Exports (tonnes)	227673	433722	329745	42248	27754
Ballast water (tonnes)	68302	130117	98924	12674	8326

Table 7: Number of vessels visiting Waterford Port 'in ballast' in 1993 and volume of ballast water discharged by commodity and LPC of vessels

Commodity	Coastal	Number of Vessels		
		Cross-Channel	EU	World
Livestock		0	0	28
Grain		3	2	0
Timber		3	7	0
Tallow		0	3	0
Frozen meat		0	0	1
Mediterranean		0	1	0
Total		6	13	29
Exports (tonnes)	6030	12499	5079*	26697*
Ballast water (tonnes)	1809	3750	1524	8009

\*Using the assumption that one animal weighed 0.75 tonnes

### United States

Carlton et al. (1994) prepared a shipping study for the U.S. Coast Guard. The study focused on the understanding of ballast water transport by the collection of port and vessel specific information. The writers visited 22 major ports and boarded vessels with

the help of the U.S.D.A. Animal and Plant Health Inspection Service (A.P.H.I.S.) inspectors. Through these efforts they were able to determine the following:

1. Ballast Water Operations: actual ballast carried versus ballast capacity, and a wide range of other data on routine ballasting, deballasting, and exchange operations in time and space.
2. Port Operations: vessel traffic patterns and unique port conditions relative to ballasting requirements, needs, and expectations.

Using the U.S. Customs/U.S. Census data, the authors estimated the amounts of ballast water and its probable source. Selected vessels greater than 250 Net Registered Tons (NRT) and greater than 500 Gross Registered Tons (GRT) were the criteria used in their study.

Of the 22 ports used in the Coast Guard Shipping Study, ports on the South Atlantic Coast and the Eastern Gulf of Mexico are the most important ports to this present study. These ports include Charleston, Savannah, Miami, Tampa, and New Orleans. The information gathered on these ports is most critical to the State of Florida. Here is some information gathered by Carlton et al. (1994) about these ports. Table 8 shows the number of ships monthly coming into the ports carrying ballast water that potentially can be dumped. Tables 9,10,11,12,13 shows the last port of call by region for ships that are in ballast from foreign ports.

Table 8: Monthly Ship Arrivals in Ballast (1991)

(From Census TM385/Vessel Entrances)

ARR= Number of vessel arrivals

BAL= Number of vessels arriving in ballast

Port Month	Charleston		Savannah		Miami		Tampa		New Orleans	
	ARR	BAL	ARR	BAL	ARR	BAL	ARR	BAL	ARR	BAL
Jan	122	5	140	4	431	173	156	41	337	100
Feb	109	2	136	9	400	164	123	40	342	116
March	115	7	131	8	535	259	138	35	352	140
April	121	4	149	7	568	248	118	34	288	85
May	124	5	158	11	504	235	136	35	314	89
June	107	3	147	8	522	273	110	30	288	81
July	126	6	153	9	513	232	110	29	355	137
Aug	124	4	154	10	539	253	106	25	333	112
Sept	133	4	143	11	484	218	112	28	277	73
Oct	130	6	157	10	492	186	113	29	333	107
Nov	105	3	151	5	488	205	128	37	314	90
Dec	117	1	138	5	508	219	126	33	366	132
Total	1433	50	1757	97	5984	2665	1476	396	3899	1262

Table 9: LPOC of Ships in Ballast from Foreign Ports (Charleston, S.C.)

REGION	FREQUENCY	% OF TOTAL FOREIGN SHIPS IN BALLAST
Northeast Atlantic	21	42
Western Central Atlantic	13	26
Mediterranean and Black Sea	7	14
Eastern Central Atlantic	3	6
Northwest Atlantic	3	6
Indian Ocean	3	6
Total	50	100

Table 10: LPOC by Region for Ships in Ballast from Foreign Ports (Savannah, GA.)

REGION	FREQUENCY	% OF TOTAL FOREIGN SHIPS IN BALLAST
Northeast Atlantic	33	34.02
Western Central Atlantic	33	34.02
Mediterranean and Black Sea	11	11.34
Northwest Pacific	10	10.31
Eastern Central Atlantic	6	6.19
Northwest Atlantic	3	3.09
Eastern Central Pacific	1	1.03
Total	97	100

Table 11: LPOC by Region for Ships in Ballast from Foreign Ports (Miami, FL.)

REGION	FREQUENCY	% TOTAL FOREIGN SHIPS IN BALLAST
Western Central Atlantic	2641	99.21
Northeast Atlantic	5	0.19
Northwest Atlantic	4	0.15
Mediterranean and Black Sea	4	0.15
Southeast Pacific	3	0.11
Eastern Central Pacific	2	0.08
Western Central Pacific	1	0.04
Eastern Central Atlantic	1	0.04
Southwest Atlantic	1	0.04
Total	2662	100

Table 12: LPOC by Region for Ships in Ballast from Foreign Ports (Tampa, FL.)

REGION	FREQUENCY	% TOTAL FOREIGN SHIPS IN BALLAST
Western Central Atlantic	171	43.40
Northeast Atlantic	90	22.84
Mediterranean and Black Sea	57	14.47
Northwest Pacific	25	6.35
Eastern Central Pacific	18	4.57
Eastern Central Atlantic	17	4.31
Southeast Pacific	7	1.78
Northwest Atlantic	4	1.02
Indian Ocean	3	0.76
Southeast Atlantic	2	0.51
Total	394	100

Table 13: LPOC by Region for Ships in Ballast from Foreign Ports (New Orleans, LA.)

REGION	FREQUENCY	% OF TOTAL FOREIGN SHIPS IN BALLAST
Western Central Atlantic	437	34.68
Northeast Atlantic	383	30.40
Mediterranean and Black Sea	252	20.00
Northwest Pacific	54	4.29
Eastern Central Pacific	46	3.65
Eastern Central Atlantic	40	3.17
Northwest Atlantic	18	1.43
Indian Ocean	16	1.27
Southeast Pacific	9	0.71
Western Central Pacific	2	0.16
Southeast Atlantic	2	0.16
Northeast Atlantic	1	0.08
Total	1260	100

According to the information gathered by Carlton et al. (1994) for the Coast Guard, it appears that three of the five ports are at some risk of contracting a foreign organism invasion due to the amount of ballast water that can be dumped. The three ports Miami, Tampa, and New Orleans are most at risk, which is crucial to the marine environment and the people living in Florida.

There is no research of ballast water and the biological contaminants that it may contain from ports in the State of Florida. Thus the present study was undertaken in order to acquire a greater understanding of the kinds of organisms that are being introduced into Florida's environment and the steps the State of Florida has taken for prevention. With this information, the risk of non-indigenous organism invasions can be easier to access.

Objectives

(1) A thorough review of the literature:

- types of cargo ships
- how organisms are transferred
- types of organisms that are transferred
- examples of organisms that have already been transferred and established in new areas
- steps that have been taken worldwide for prevention.

(2) Generate local data from ports on ballast water contents, primarily microbial.

## MATERIALS AND METHODS

Ballast water samples were taken from various sites around the State of Florida. The samples were then subjected to several different analyses, which are listed in detail below. The basis of these analyses is to see if they correspond with existing data.

### Source of Ballast Water

Ballast water was collected from four primary sources. These sources are Mayport in Jacksonville, FL, St. Petersburg Harbor, FL, Port of Tampa in Tampa Bay, FL, and Port Manatee in Palmetto, FL. All provided ballast water, however, Port Manatee provided the most help and effort in attempting to acquire the samples. Dr. William Tiffany and Mr. John Ballaron collected specimens from Port Manatee to be used in this study.

### Collection of Ballast Water

The ballast water was collected under the most sterile conditions possible. A percentage of the cargo ships had ballast tanks that could be opened, which allowed the water to be dipped out with sterile containers. Other cargo ships did not have ballast tanks that could be opened, so the water was pumped out of small ports in the hull. The samples were then put on ice and sent to the lab for analysis.

### Microbiological Analyses

Microbiological analysis was conducted immediately after the samples were received. The water samples were serially diluted into 99ml of phosphate buffer water to  $10^{-5}$ . Most probable number (MPN) tubes, done in triplicate, were first inoculated. The

MPN tubes included 9.0ml alkaline peptone water (APW) at 2.5% NaCl and pH=8.4, and were inoculated with 1ml from the dilution. APW is an enrichment medium in which the *Vibrio* genus can grow quite well due to the high salt concentration and the high pH (Dixon,1996). The tubes were diluted out to  $10^{-5}$ . After inoculation, the tubes were incubated at 37°C for 8-12 hours and were then checked for growth/turbidity. Growth and or turbidity are considered a positive result. The positive and/or negative tubes make up a three-digit code number, which can be cross-referenced in a MPN table. This three digit code provides a statistical count for bacteria (Dixon,1996). The positive tubes were then streaked onto thiosulfate-citrate-bile salts-sucrose agar (TCBS) and colistin-polymixin-cellobiose agar (CPC) (Dixon, 1996). TCBS agar is selective for “*Vibrio*-like” bacteria and CPC is selective for *Vibrio vulnificus* and *Vibrio cholerae* (Dixon, 1996). The streaked TCBS plates were incubated for 24 hours at 37°C (Dixon, 1996), and observed for growth. Yellow colonies were streaked on gelatin agar with 2% salt and gelatin agar with no salt. Growth on gelatin agar with 2% salt is presumptive for *Vibrio parahemolyticus*. CPC plates were incubated for 24 hours at 42°C and observed for growth. Most bacteria are inhibited at 42°C, which is the reason for such a high incubation temperature.

One ml samples from the serial dilution was pour plated on total plate count agar (PCA) in duplicate. This agar gave an assessment of the total aerobic plate count. Further, 1ml samples from the serial dilution was inoculated into A1 broth tubes, 5 tubes per dilution, and incubated at 44.5°C water bath. Like the APW MPN, the positive A1 tubes, gas and growth, also can be used as an MPN to give a count of the presumptive total coliform number. One ml samples from positive A1 tubes were transferred to



corresponding EC tubes and BG tubes. The EC tubes were incubated at 44.5°C for total fecal coliforms and BG was incubated at 37°C for total coliforms. The positive tubes were counted as a MPN to give a total count of *E. coli*.

#### Invertebrate Analysis

Several samples were magnified under a microscope for the presence of invertebrates and other single celled organisms. In addition, all samples were filtered in four sieves, with spacing ranging from 0.1-1 cm in diameter.

#### Turbidity Analysis

Several samples were run through optical density spectrophotometry at 540nm in order to acquire their turbidity readings.

#### Other Analyses

Several samples were sent to the University of Florida's Soils Department in order for them to run specific tests. Ca, Mg, K, P, Zn, Cu, Mn, Al, Fe, Na, B, Pb, Ni, Ba, Mo, Si, and Cd were analyzed through inductively coupled argon plasma spectroscopy (ICP), Thermo Jarrell Ash (TJA) Corporation, Franklin, MA. Cl, ammonia, and nitrate were analyzed through air segmented automated spectrophotometry, R.F.A. 300 series, Alpkem, College Station, Texas. Ph and electrical conductivity were also analyzed in certain samples.

## RESULTS AND DISCUSSION

The results of the four area ports where ballast water samples were taken are below. Table 14 lists the results of ballast water taken from Mayport, FL, tables 15 and 16 lists the results of ballast water taken from St. Petersburg Harbor, FL, table 17 lists the results of ballast water taken from Tampa Bay, FL, and Table 18 lists the results of ballast water taken from Port Manatee, FL.

### Microbiology Analysis Results

#### Mayport, FL

Table 14: Microbiological results of Ballast Water from Mayport, FL.  
2 Samples (S.E. Asia)

	S.E. Asia	S.E. Asia
Fecal Coliforms cfu/ml	>1600	>1600
A. Plate Count cfu/ml	$1.2 \times 10^5$	$1.8 \times 10^6$
MPN Vibrio Like cfu/ml	$1.2 \times 10^4$	$2.2 \times 10^4$
Non01 V. cholera	+	+
V. vulnificus	+	+
V. para.	+	+
Invert. Anal.	-	+
Vertebrate Anal.	+	+

#### St. Petersburg Harbor, FL (Puerto Rican Samples)

Table 15: Microbiological results of Ballast Water from St. Petersburg Harbor, FL.  
4 Samples (Puerto Rico)

	Puerto Rico	Puerto Rico	Puerto Rico	Puerto Rico
Fecal Coliforms cfu/ml	>1600	>64	<32 N.D.	<32 N.D.
A. Plate Count cfu/ml	$22 \times 10^4$	$1.8 \times 10^5$	$1.1 \times 10^3$	$1.1 \times 10^3$
MPN Vibrio Like cfu/ml	64	242	32	32
Non01 V. cholera	+	+	+	+
V. vulnificus	+	+	+	+
V. para.	+	+	+	+
Invert. Anal.	-	-	-	-
Vertebrate Anal.	-	-	-	-

St. Petersburg Harbor, FL (Caribbean Samples)Table 16: Microbiological results of Ballast water taken from St. Petersburg, FL.  
15 Samples (Caribbean)

Sample	F.C. cfu/ml	A.P.C. cfu/ml	Vibrio- like	V. Para	V. vul	Invert. Anal.	Vert. Anal.
1	64	10000	+	+	+	-	-
2	-	100000	+	+	+	+	-
3	32	10000	+	+	+	-	-
4	-	1000	+	+	+	-	-
5	32	1000	+	+	+	+	-
6	64	10000	+	+	+	-	-
7	242	10000	+	+	+	-	-
8	-	10000	+	+	+	-	-
9	-	100000	+	+	+	+	-
10	-	1000	+	+	+	-	-
11	242	1000	+	+	+	-	-
12	64	1000	+	+	-	+	-
13	32	10000	+	+	-	-	-
14	32	1000	+	+	-	-	+
15	-	1000	+	+	-	+	+

Tampa Bay, FLTable 17: Microbiological results of Ballast Water from Tampa Bay, FL.  
3 Samples (Puerto Rico, Venezuela, Chicago)

	Puerto Rico	Venezuela	Chicago
Fecal Coliforms cfu/ml	>1600	>1600	-
A. Plate Count cfu/ml	850	59000	425
MPN Vibrio Like cfu/ml	4	8	4
Non01 V. cholera	-	-	-
V. vulnificus	+	+	-
V. para	+	+	-
Invert. Anal.	-	-	-
Vertebrate Anal.	-	-	-

Port Manatee, FLTable 18: Microbiological and Metal results of Ballast Water from Port Manatee, FL.  
4 Samples (Jacksonville, Caribbean, New Orleans, Maryland)

	Jacksonville	Caribbean	New Orleans	Maryland
Fecal Coliforms cfu/ml	130	17	23	7.3
A. Plate Count cfu/ml	1640	449	83	475
MPN Vibrio Like cfu/ml	4	4	-	-
Non O1 V. cholera	-	-	-	-
V. vulnificus	+	+	-	-
V. para.	+	+	-	-
Invert. Anal.	-	-	-	-
Vertebrate Anal.	-	-	-	-
Turbidity (450 nm)	0.007	0.004	0.003	0.004
PH	8.27	8.29	8.0	6.8
E. Conductivity	42.6	39.7	0.6	65.6
Ca (mg/L)	348	330	51.3	360
Mg (mg/L)	1110	1040	17.2	1500
K (mg/L)	434	412	4.2	431
P (mg/L)	-	0.1	0.05	-
Zn (mg/L)	0.06	0.12	-	0.01
Cu (mg/L)	0.02	-	-	-
Mn (mg/L)	-	-	-	0.11
Al (mg/L)	-	-	-	-
Fe (mg/L)	-	-	-	0.01
Na (mg/L)	563	5340	33.8	11400
B (mg/L)	4.32	4.06	0.10	4.45
Pb (mg/L)	-	0.01	-	-
Cl (mg/L)	18500	17000	30.5	21000
Na4-N	0.8	-	-	-
NO3-N	-	-	2.5	-
Ni (mg/L)	-	-	-	-
Ba (mg/L)	0.01	0.01	0.05	0.01
Mo (mg/L)	0.04	0.03	-	0.03
Si (mg/L)	-	-	3.6	-
Cd (mg/L)	-	-	-	-

In several samples, the fecal coliform load was greater than 1600 cfu/ml. This high value could be due to several reasons. The cargo ships could have exchanged ballast

in waters that were high in fecal coliforms. Also, the cargo ships could have cross-contaminated between bilge tanks and ballast tanks. Bilge tanks consist of the ship's sewage lines and unused oil lines. Some samples contained one or more *Vibrio* species while others contained none. The samples that contained one or more *Vibrio* species came from South East Asia, Puerto Rico, Venezuela, and Jacksonville. These were the last port of call; however, the cargo ships did not necessarily pick up ballast from these areas. The ballast might not have been exchanged for over a month, so the water could have come from any previous port of call. Further, there is no documentation where the ships exchanged ballast. In several samples from St. Petersburg, FL., *V. vulnificus* did not grow whereas *V. parahaemolyticus* did. A reason for the growth is *V. parahaemolyticus* can tolerate higher salinities than other *Vibrio* species.

Four samples of ballast water were taken at Port Manatee were analyzed for metal content. The pH of three was 8.0 or higher. The sample from Maryland had a pH of 6.8. It also had the highest values of chlorine, magnesium, sodium, and calcium. It also had the highest electrical conductivity. This is likely due to the greater amount of metals it contained as compared to the other three samples. The average amount of chloride found in ocean water is 19.344g/kg (Riley and Chester, 1981). This is around the range of samples taken from Jacksonville, the Caribbean, and Maryland; however, the sample taken from New Orleans, however, was quite higher, which suggests that chloride might have been added to the water. Further evidence reveals that the microbial load is lower in this sample than in the other three. All other values fall within the ranges described in the literature (Riley and Chester, 1981).

## CONCLUSION AND RECOMMENDATIONS

Organisms are being transferred from other areas to the Florida coast. In order to determine the amount and frequency that non-indigenous organisms are being introduced, more samples need to be analyzed. Proper records should be kept in order to determine the exact origin of the ballast water on board. The U.S. Coast Guard established voluntary guidelines in April, 1998. These guidelines encourage ship crews to keep records of how much ballast water is on board, the last area where the ship ballasted, and the time of ballast. Temperature of ballast water should be recorded as well. Having knowledge of these parameters might lead to a better understanding of handling the introduction problems around Florida. The U.S. Coast Guard also encourages the ships to ballast in deeper ocean water in order for the ballasted water to be around 32.4ppt salinity. Very few coastal organisms survive at this salinity (O'Shea, 1998).

Facilities such as water purification and disinfection plants are expensive, but are options for the future. On board mechanisms, such as filtration and heat treatment, are also possible future options. This would require ship redesign. Ships also need to be designed so that the bilge water does not contaminate the ballast tanks. This will reduce the amount of microorganisms, such as fecal coliforms, in ballast water. Port Manatee provides fresh treated water for potable or ballast purposes to reduce non-indigenous organism introduction. According to staff members of Port Manatee, however, this supply has have never been used for ballast due to the expense. For now, ballasting in

deep ocean water is the best known practice for reduction of non-indigenous organism introductions.

For future research, there should be a risk assessment executed in order to determine which area ports are the most at risk for invasion from non-indigenous species. Records of foreign and domestic ships coming into ports in ballast with the potential to release water should be taken. Further, legislative acts should be passed in order for the collection of ballast water to be assessable. Ships coming into area ports should understand that there is a chance that a sample of ballast water will be asked of them and they should comply.

## LITERATURE CITED

- Anderson, I. 1992. End of the line for deadly stowaways. *New Scientist*. 24: 12-13.
- Anderson, I. 1993. Aliens slip through international "safety net". *New Scientist*. 3: 5.
- Anon. 1992. Marine Environment Protection Committee recommendation: Australia's Response. *Marine Pollution Bulletin*. 24(2): 117-118.
- Anon. 1993a. Zebra mussel wandering. *World Aquaculture*. 24(3): 86-89.
- Anon. 1993b. Resolution A.774(18). Guidelines for preventing the introduction of unwanted aquatic organisms and pathogens from ships' ballast water and sediment discharges. International Maritime Organization; Australia: 14.
- Anon. 1997a. Fish invader threatens Great Lakes ecosystem. The Associated Press.
- Anon. 1997b. N.C. studying foreign ships' bilge. The Associated Press.
- Bederman, D.J. 1991. International control of marine "pollution" by exotic species. *Ecology Law Quarterly*. 18: 677-717.
- Bolch, C.J. and Hallegraeff, G.M. 1993. Chemical and physical treatment options to kill toxic dinoflagellate cysts in ships' ballast water. *Journal of Marine Environmental Engineering*. 1: 23-29.
- Burkholder, J.M., Noga, E.J., Hobbs, C.H. and Glasgow, H.B. Jr. 1992. New "phantom" dinoflagellate is the causative agent of major estuarine fish kills. *Nature*. 358: 407-410.
- Carlton, J.T. 1985. Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water. *Oceanography and Marine Biology Annual Review*. 23: 313-371.
- Carlton, J.T. 1987. Patterns of transoceanic marine biological invasions in the Pacific Ocean. *Bulletin of Marine Sciences*. 41(2): 452-465.
- Carlton, J.T. 1989. Man's role in changing the face of the ocean: Biological invasions and implications for conservation of near-shore environments. *Conservation Biology*. 3(3): 265-273.



- Carlton, J.T. and Geller, J.B. 1993. Ecological roulette: the global transport of non-indigenous marine organisms. *Science*. 261: 78-82.
- Carlton, J.T., Reid, D.M. and van Leeuwen, H. 1994. Shipping study: The role of shipping in the introduction of non-indigenous aquatic organisms to the coastal waters of the United States (other than the Great Lakes) and an analysis of control options. National Sea Grant College Program / Connecticut Sea Grant Project R/ES-6; Mystic, CT: 1-213.
- Cognetti, G. and Curini-Galletti, M. 1993. Biodiversity Conservation Problems In the Marine Environment. *Marine Pollution Bulletin*. 26(4): 179-183.
- Dixon, D.W. 1996. The Influence Of Gamma Radiation Upon Shellstock Oysters, and Culturable and Viable But Not Nonculturable Vibrio Vulnificus. Doctoral Dissertation, University of Florida, Gainesville.
- Epstein, P.R. 1993. Algal Blooms In the Spread and Persistence of Cholera. *Biosystems*. 31(2-3): 209-221.
- Gosliner, T.M. 1995. Introduction and spread of *Philine auriformis* (Gastropoda: Opisthobranchia) from New Zealand to San Francisco Bay and Bodega Harbor. *Marine Biology*. 122: 249-255.
- Hallegraeff, G.M. 1995. Transport of toxic dinoflagellates via ships' ballast water: An Interim Review, ICES Conference, Aalborg: Theme Session On Ballast Water: CM 1995/0: 1-11.
- Hallegraeff, G.M. and Bolch, C.J. 1991. Transport of toxic dinoflagellate cysts via ships' ballast water. *Marine Pollution Bulletin*. 22(1): 27-30.
- Hallegraeff, G.M. and Bolch, C.J. 1992. Transport of diatom and dinoflagellate resting spores in ships' ballast water: Implications for Plankton biogeography and aquaculture. *Journal of Plankton Research*. 14(8): 1067-1084.
- Hedgepeth, J.W. 1993. Foreign invaders. *Science*. 261: 34-35.
- Herbold, B. and Moyle, P.B. 1986. Introduced species and vacant niches. *American Naturalist*. 128(5): 751-760.
- Hutchings, P. 1992. Ballast Water Introductions of Exotic Marine Organisms Into Australia: Current Status and Management Options. *Marine Pollution Bulletin*. 25(5-8): 196-199.
- Leppakoski, E.J. 1991. Introduced species-resource or threat in brackish-water seas? Examples from the Baltic and the Black Sea. *Marine Pollution Bulletin*. 23: 219-223.

- Lewis, E.V. and O'Brien, R. 1965. Ships: The floating box. Life Science Library; Time Inc., New York: 8-16.
- Locke, A., Reid, D.M., van Leeuwen, H.C., Sprules, W.G. and Carlton, J.T. 1993. Ballast water exchange as a means of controlling dispersal of freshwater organisms by ships. Canadian Journal of Fisheries and Aquatic Sciences. 50(10): 2086-2093.
- Luczak, C., Dewarumez, J.M. and Essink, K. (1993). First record of the American Jackknife Clam *Ensis directus* on the North Sea. Journal of the Marine Biological Association of the U.K. 73(1): 233-235.
- Macleod, L. 1995. Risks associated with the uncontrolled discharge of ships' ballast and the legislative controls available in Scotland. Journal of the Civil Western Environmental Management. 9: 173-178.
- Marsden, J.E. 1993. Responding to aquatic pest species: Control or management? Fisheries. 18(1): 4-5.
- McCarthy, S.A. and Khambaty, F.H. 1994. International dissemination of epidemic *Vibrio cholerae* by cargo ship ballast water and other non-potable waters. Applied and Environmental Microbiology. 60(7): 2597-2601.
- McCarthy, S.A. and Miller, A.L. 1994. Effect of three biocides on Latin American and Gulf Coast Strains of toxigenic *Vibrio cholerae* 01. Journal of Food Protection. 57(10): 865-869.
- McMahon, R.F. and Tsou, J.L. 1990. Impact of European zebra mussel infestation to the electric power industry. Annual Meeting of the American Power Conference. Chicago. April: 23-25.
- Moyle, P.B. 1991. Ballast water introductions. Fisheries. 16(1): 4-6.
- Newman, S. 1998. Jellyfish invasion; Earthweek: A diary of the planet, Universal Press Syndicate. May 19.
- Omori, M., van der Spoel, S. and Norman, C.P. 1994. Impact of human activities on pelagic biogeography. Progress in Oceanography. 34: 211-219.
- O'Shea, S. (1998). Coast guard proposes national ballast water regulations. Louisiana Coastal Law. 72: 1-8.

- Rigby, G.R., Stevenson, I.G., and Hallegraeff, G.M. 1993. The transfer and treatment of shipping ballast waters to reduce the dispersal of toxic dinoflagellates. In: T.J. Smayda and Y. Shimizu (eds.), *Toxic Phytoplankton Blooms in the Sea*. Proceedings of the Fifth International Conference on Toxic Marine Phytoplankton. Elsevier, Amsterdam: 169-176.
- Rigby, G.R., Taylor, A.H., Hallegraeff, G.M., and Mills, P. 1995. Progress in research and management of ships' ballast water to minimize the transfer of toxic dinoflagellates. *Harmful Marine Algal Blooms*. 1: 821-824.
- Riley, J.P. and Chester, R. 1981. *Introduction to Marine Chemistry*, Academic Press, Inc., New York.
- Scholin, C.A. and Anderson, D.M. 1994. Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). I. RFLP analysis of SSU rRNA genes. *Journal of Phycology*. 30: 744-754.
- Scholin, C.A., Herzog, M., Sogin, M., and Anderson, D.M. 1994. Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. sequence analysis of a fragment of the LSU rRNA gene. *Journal of Phycology*. 30: 999-1011.
- Sheehan, J. 1995. Ships' ballast water as a vector for the introduction of non-indigenous species: Implications for Ireland. Master's Thesis, Trinity College, Dublin, Ireland.
- Suchanek, T.H. 1994. Temperate coastal marine communities: Biodiversity and threats. *American Zoologist*. 34(1): 100-114.
- Williams, R.J., Griffiths, F.B., van der Wal, E.J., and Kelly, J. 1988. Cargo vessel ballast water as a vector for the transport of non-indigenous marine species. *Estuarine, Coastal and Shelf Science*. 26: 409-420.
- Young, C. 1994. Hidden threat in ballast water. *Western Fisheries*: 40.

## BIOGRAPHICAL SKETCH

Linda Asbury Lore was born in Asheville, North Carolina on September 5, 1966. She graduated from Oak Hall Private School in 1985 and enrolled at Santa Fe Community College in the summer of 1985. Linda received an Associate of Arts degree in 1989 and was accepted in to the College of Business Administration at the University of Florida. She went back to Santa Fe Community College in 1991 and returned to the Food Science and Human Nutrition Department at the University of Florida in 1993. There she received a dietetics and food science degree in 1995 and was accepted into the food science graduate program in 1995. Linda is currently employed by Columbia North Florida Hospital Department of Microbiology.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.



Gary E. Rodrick, Chair  
Professor of Food Science and  
Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.



Murat O. Balaban  
Professor of Food Science and  
Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.



William J. Tiffany III  
Director of Environmental Affairs  
Port Manatee, Florida

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.



John A. Cornell  
Professor of Statistics

This thesis was submitted to the graduate faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Master of Science.

December, 1998

  
Dean, College of Agriculture

---

Dean, Graduate School